

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Currently amended) A method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs comprising:

a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;

b) synthesizing a second strand of cDNA by contacting under conditions conducive to a thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 55°C to 70°C ~~45°C to 80°C~~, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA; and

c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced.

2. (Currently amended) The method of claim 1 wherein said incubation temperature is from 60°C to 65°C ~~55°C to 70°C~~.

3. (Canceled)

4. (Previously presented) The method of claim 1 wherein the conditions for synthesizing the second strand of cDNA further comprise an incubation time of from one to sixty minutes.

5. (Previously presented) The method of claim 1, which further comprises labeling the transcribed cRNA with a fluorescent, radioactive, enzymatic, hapten, biotin, or digoxigenin label.

6. (Previously presented) The method of claim 1, wherein the thermostable Bst DNA polymerase large fragment is present in a concentration of from 0.012 to 1.3 units/ μ l and the thermostable RNase H is present in a concentration of from 0.0031 to 1.3 units/ μ l.

7. (Previously presented) The method of claim 5, wherein the label is fluorescent.

8. (Original) The method of claim 7 wherein the fluorescent label is fluorescein isothiocyanate, lissamine, Cy3, Cy5, or rhodamine 110.

9. (Original) The method of claim 7, wherein a first aliquot of the cRNA is labeled with a first fluorophore having a first emission spectrum, and a second aliquot of the cRNA is labeled with a second fluorophore with a second emission spectrum differing from that of the first emission spectrum.

10. (Original) The method of claim 9, wherein the first fluorophore is Cy3 and the second fluorophore is Cy5.

11. (Original) The method of claim 1 further comprising, after the transcribing step, determining the presence or absence of a pre-selected target mRNA in said sample.

12. (Previously presented) The method of claim 1, wherein the conditions for synthesizing the second strand of cDNA further comprise an incubation time of from five to thirty minutes.

13. (Previously presented) The method of claim 1, wherein the mRNA is extracted from at least one cell of interest, and further comprising contacting the cRNA produced in step (c) with an array containing one or more species of polynucleotide positioned at pre-selected sites on the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said one or more species of polynucleotide and said cRNA.

14. (Currently amended) A method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, said first sample and said second sample each containing a plurality of different mRNAs from one or more cells, comprising:

- a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;
- b) synthesizing a second strand of cDNA by contacting, under conditions conducive to thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 55°C to 70°C 45°C to 80°C, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA;
- c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced;
- d) labeling the cRNA produced in step (c) with a first label;
- e) repeating steps (a) – (c) with said second sample;
- f) labeling the cRNA produced in step (e) with a second label distinguishable from said first label;
- g) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA;
- h) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA; and

i) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample.

15. Cancelled.

16. (Previously presented) The method of claim 14 wherein said sample contains total RNA or total mRNA from mammalian cells.

17. (Currently amended) The method of claim 14 wherein the incubation temperature is from 60°C to 65°C 55°C to 70°C.

18. (Original) The method of claim 14 wherein said first label is Cy3 and said second label is Cy5.

19. (Original) The method of claim 14 wherein said detecting or measuring steps (g) and (h) are carried out by a method comprising contacting said cRNA with an array containing one or more species of polynucleotide positioned at pre-selected sites on the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA.

20. (Previously presented) The method of claim 19 wherein the array comprises a support with at least one surface and more than one different polynucleotides, each different polynucleotide comprising a different nucleotide sequence and being attached to the surface of the support in a different, selected location on said surface.

21. (Previously presented) The method of claim 19 wherein the array has at least 1,000 polynucleotide probes per square centimeter.

22. (Original) The method of claim 14 wherein in steps (g) and (h), the steps of contacting the cRNA labeled with said first label with said polynucleotide probe, and

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contacting the cRNA labeled with said second label with said polynucleotide probe, are carried out concurrently.

23. (Original) The method of claim 14 wherein said first sample contains mRNAs from cells that are pathologically aberrant and wherein said second sample contains mRNAs from cells that are not pathologically aberrant.

24-26. Cancelled.